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Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed, USA

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Abstract Understanding the quantity and quality of dissolved organic matter (DOM) in potential watershed sources is critical for explaining and quantifying the exports of DOM in stream runoff. Here, we examined the concentration and quality of DOM for ten watershed sources in a 12 ha forested catchment over a two-year period. DOM composition was evaluated for: throughfall, litter leachate, soil water (zero and tension), shallow and deep groundwater, stream water, hyporheic zone, and groundwater seeps. DOM quality was measured using a suite of optical indices including UV–visible absorbance and PARAFAC modeling of fluorescence excitation-emission matrices (EEMs). DOM concentrations and quality displayed a pronounced trend across watershed sources. Surficial watershed sources had higher DOM concentrations and more humic-like DOM with higher molecular weight whereas deeper groundwater sources were rich in % protein-like fluorescence. The greater % contribution of protein-like fluorescence in groundwater suggested that a larger fraction of groundwater DOM may be bioavailable. DOM for wetland groundwater was more aromatic and humic-like than that at the well-drained riparian location. Principal component analyses (PCA) revealed that the differences in surficial watershed compartments were dictated by humic-like components while groundwater sources separated out by % protein-like fluorescence. Observations from optical indices did not provide any conclusive evidence for preferential association of dissolved organic carbon (DOC) or dissolved organic nitrogen (DON) with any particular DOM quality pools.

Keywords DOC · DON · Organic matter · Aromatic · Bioavailable · Fluorescence · UV absorbance · Excitation emission matrices (EEMs)

Introduction

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) play key roles in the biogeochemistry of ecosystems. DOC plays an important role in the acid–base chemistry of acid sensitive freshwater systems (Herczeg et al. 1985); affects the complexation, solubility and mobility of metals such as aluminum and mercury (Driscoll et al. 1988); is linked to the formation of potentially

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carcinogenic trihalomethanes when surface water is chlorinated for drinking (Nokes et al. 1999; Siddiqui et al. 1997); and attenuates UV radiation and thus provides protection to aquatic biota (Williamson and Zagarese 1994). Similarly, DON constitutes a significant portion of the total N flux for some ecosystems (Campbell et al. 2000; Hedin et al. 1995; Neff et al. 2003); and can become bioavailable for estuarine plankton (Seitzinger et al. 2002). Thus, understanding the dynamics of C and N components of dissolved organic matter (DOM) is of considerable environmental significance.

Previous research indicates that concentrations of DOC and DON may differ considerably among watershed sources (Goller et al. 2006; Hagedorn et al. 2001; Inamdar and Mitchell 2007; Michalzik et al. 2001) which may influence the export of DOM from watersheds (Campbell et al. 2000; Inamdar and Mitchell 2006, 2007). In addition, C and N-rich components of DOM may differ with respect to mobility, degradability, and bioavailability (Fellman et al. 2008; Hagedorn et al. 2001; Kaushal and Lewis 2003; McDowell 2003; Petrone et al. 2008). Some studies suggest that these differences may stem from the differential behavior of DOM constituents with varying C:N ratios (Qualls and Haines 1991, 1992; Yano et al. 2005). These DOM constituents include hydrophobic and hydrophilic and/or aromatic and non-aromatic fractions (Qualls and Haines 1991, 1992). Hydrophobic fractions include humic and aromatic DOM moieties, tannins and polyphenols, and complexed amino acids while hydrophilic fractions could be composed of carbohydrates, small carboxylic acids, free proteins and peptides (Qualls and Haines 1991; Yu et al. 2002).

Dissolved organic matter (DOM) concentrations are typically highest in surficial watershed sources like throughfall and the forest floor and then decrease dramatically as DOM percolates through the mineral soil profile (Aitkenhead-Peterson et al. 2003; Kalbitz et al. 2000; Michalzik et al. 2001). This has been attributed to the preferential sorption of hydrophobic compounds in soils (Jardine et al. 1989; Kaiser and Zech 1998, 2000) whereas the hydrophilic fractions have been found to be more mobile and remain in solution (Kaiser and Zech 1998; Ussiri and Johnson 2004). The mobile hydrophilic DOM constituents have also been reported to be N-rich while the hydrophobic constituents were found to contain more C (Kaiser and Zech 1998; Qualls and Haines 1991; Ussiri and Johnson 2004). Conversely, others have reported that N-containing DOM may also be hydrophobic (McKnight et al. 1992; Yu et al. 2002). Yu et al. (2002) attributed the hydrophobic behavior to complexation of N rich amino compounds or proteins/peptides with polyphenols and humic substances.

In addition to sorption, biotic processes such as microbial immobilization and mineralization may also influence DOM dynamics (Aitkenhead-Peterson et al. 2003). Here too, important differences have been noted between the relative bioavailability of C-rich and N-rich DOM (Kaushal and Lewis 2003, 2005; Petrone et al. 2008; Wiegner and Seitzinger 2001). Petrone et al. (2008) attributed this difference to the greater lability of N-rich hydrophilic fraction compared to the C-rich hydrophobic portion of DOM. The extent of alteration of DOM by processes such as sorption, microbial consumption, or photolysis (Aitkenhead-Peterson et al. 2003) may eventually be dictated by the hydrologic flow paths taken by DOM as it moves through the watershed. Surficial flow paths may limit the contact time with mineral soil thus limiting the removal of DOM by sorption; while fast flow paths such as surface runoff or macropore flow may reduce the residence time and therefore the extent of microbial processing.

These observations suggest that depending on the hydrologic flowpaths, sorption, and microbial processes, the amount and quality DOM may vary considerably across watershed sources, both in space and time. Understanding the quantity and quality of DOM in watershed sources (e.g., throughfall, forest floor, soil water, and groundwater) is critical to assessing the exports of DOM. The relative contributions of surficial and groundwater sources to runoff are expected to differ between storm and non-storm conditions (Inamdar and Mitchell 2006) and thus knowledge of DOM for individual watershed sources will provide a better quantification of stream/catchment exports of DOM. The recent availability of innovative spectrofluorometric tools such as ultra violet (UV–visible) absorbance (Spencer et al. 2008, 2009; Weishaar et al. 2003) and fluorescence (Fellman et al. 2008, 2009; Hood et al. 2005, 2006; Jaffé et al. 2008; McKnight et al. 2001) allow for rapid characterization of DOM constituents and are especially suitable for catchment-scale studies that generate large number of samples. These indices also provide important insights into DOM mobility and bioavailability and include: specific ultra-violet absorbance (SUVA, Weishaar et al. 2003) and spectral slope ratio (S_R, Helms et al. 2008) from UV absorbance; and humification index (HIX) (Ohno 2002), fluorescence index (FI, Cory and McKnight 2005), % protein-like fluorescence (Fellman et al. 2008, 2009) derived from fluorescence-based excitation emission matrices (EEMs, Cory and McKnight 2005; McKnight et al. 2001).

Our objective in this study was to use a suite of these optical indices to characterize the DOM quality for all watershed sources that likely contribute to watershed DOM exports. Specific attention was paid to investigating how the relative concentrations of DOC and DON differed for watershed sources and their relationship with optically derived DOM quality indices. Sampling was performed over a 2-year period in a 12 ha forested catchment located in the Piedmont province of Maryland, USA. Watershed sources that were sampled included: throughfall, litter leachate (O horizon), wetland soil water, shallow and deep groundwaters from wetland and riparian landscape positions, surface seep water, hyporheic water and streamwater. These sources were selected because they were a continuum of potential sources that contributed to stream runoff during storm and non-storm conditions at this site. DOM quality for these watershed sources was indirectly characterized using multiple spectrofluorometric indices. Specific questions that were addressed include: (a) How do DOM concentrations and quality vary across watershed sources and what processes or factors explain their variation? (b) What do spectrofluorometric DOM quality indices indicate about the mobility and bioavailability of DOM in the watershed? and (c) Are DOC or DON concentrations correlated with specific DOM constituents as determined from optical indices? The novel aspect of this work is the catchment-scale assessment of DOM for multiple watershed sources through the use of a suite of optical indices.

Site description and methods

Site description

The study catchment (12 ha) is located within the Fair Hill Natural Resources Management Area (NRMA) (39°42′N, 75°50′W) in Cecil County, MD (Fig. 1) and is part of the Big Elk Creek drainage basin. The basin lies within the Piedmont physiographic region and eventually drains into the Chesapeake Bay. Cecil County has a humid, continental climate with well-defined seasons. The maximum daily mean temperature (1971–2000) was 24.6°C (July) and the daily minimum was −0.6°C (January), with a mean annual temperature of 12.2°C. For this same period, mean annual precipitation in this region was 1231 mm with ~350 mm occurring as snowfall in winter (Maryland State Climatologist Office 2008). Late summer (August–September) tends to be driest period of the year while late spring (May–June) is the wettest.

The study area is underlain by the Mt. Cuba Wissahickon formation and includes pelitic gneiss and pelitic schist with subordinate amphibolite and pegmatite (Blackmer 2005). The soils in the study area belong to the Glenelg series, which consists of deep, well-drained, nearly level to moderately steep soils. On the hillslopes soils are coarse loamy, mixed, mesic Lithic Dystrudepts while in the valley bottoms seasonal water saturation leads to the formation of Oxyaquic Dystrudepts. Elevation ranges from 252 to 430 m above mean sea level.

Vegetation in the study catchment consists of deciduous forest with pasture along the catchment periphery. Dominant tree species are *Fagus grandifolia* (American beech), *Liriodendron tulipifera* (yellow poplar), and *Acer rubrum* (red maple). Based on a survey of canopy trees (>10 cm diameter at breast height), there is a stand density of 225 trees ha^{-1}, a stand basal area of 36.8 m^2 ha^{-1}, a mean diameter at breast height (dbh) of 40.8 cm, and a mean tree height of 27.8 m (Levia et al. 2010).

Watershed instrumentation and sampling

Streamflow discharge was measured at the 12 ha catchment outlet (Fig. 1) using a Parshall flume with 15-cm throat-width along with a Global Water (Inc.) logger and pressure transducer. Groundwater elevations were recorded at five locations in the valley-bottom at 30-min intervals using Global Water loggers (Inc.). Groundwater logging wells consisted of PVC pipes (5 cm diameter) ~2 m below the ground surface that were continuously slotted from a depth of 0.3 m below the soil surface.

Manual grab sampling was performed every 3 weeks during non-storm periods from July 2007

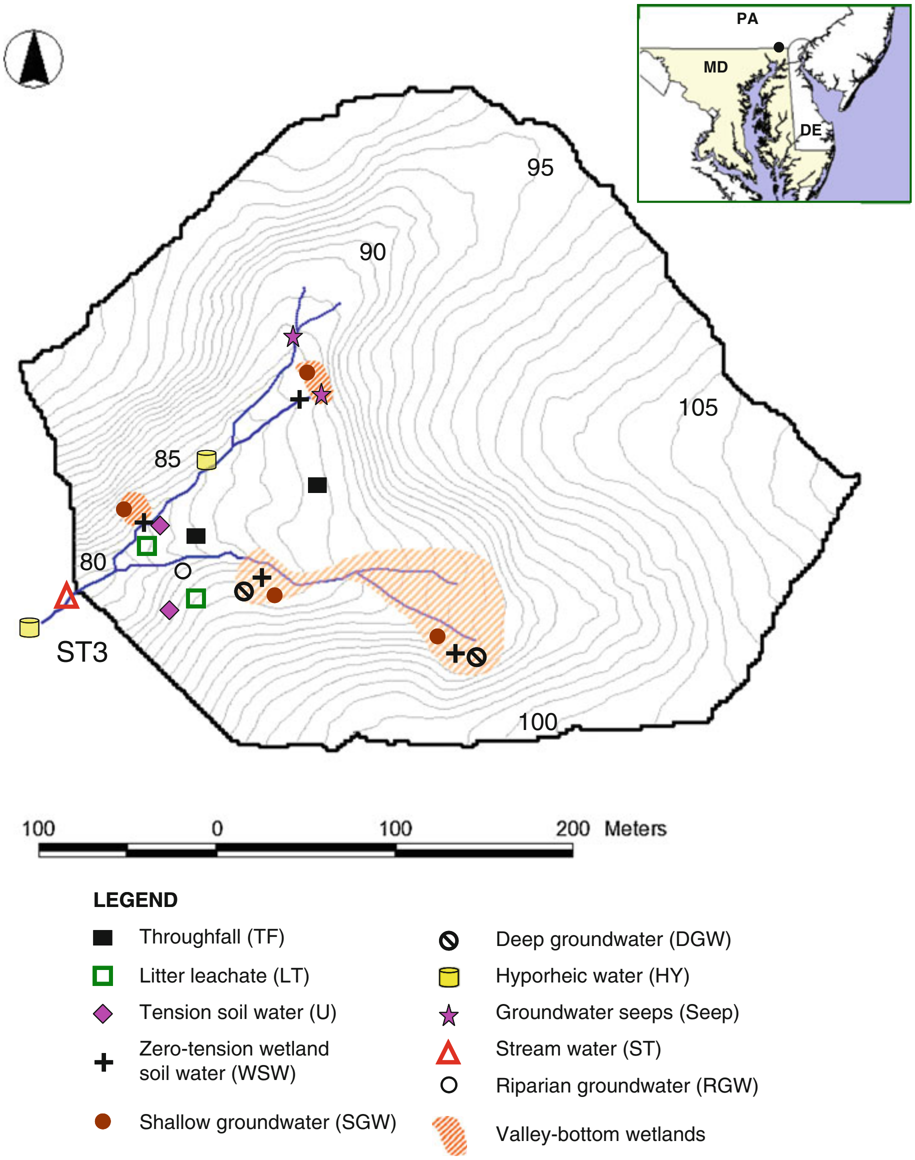


Fig. 1 Location of the study site in the Piedmont region of northeastern Maryland (*inset* indicated by *filled circle*) and the sampling locations within the 12 ha study watershed

to July 2009. While most sampling devices were installed in the spring/summer of 2007, devices for deep groundwater and hyporheic water were installed in October 2007 and April 2008, respectively. Samples collected included: streamwater (ST); zero-tension wetland soilwater (WSW); tension soilwater (U); wetland shallow and deep groundwater (SGW and DGW, respectively), riparian groundwater (RGW), groundwater seeps (Seep) and hyporheic water (HY) (Fig. 1). Streamwater samples were collected at the outlet of the 12 ha catchment outlet. Wetland soilwater was sampled at four sites (Fig. 1) in the valley-bottom wetlands using zero-tension lysimeters which consisted of screened 5 cm diameter PVC pipes that were inserted at a 45° angle to a depth of 30 cm thus collecting free pore soil water from the top 30 cm of the soil profile. Valley-bottom wetlands were variably saturated over the year and were wettest in late spring (May–June) and driest in late summer (August–September). Tension soilwater samples were collected using two nests of two suction cup tension lysimeters (Soilmoisture Equipment Corp.) each. One nest was located at the edge of a valley-bottom saturated area while the other was located at a drier location at the bottom of a hillslope (Fig. 1). At each nest, one tension lysimeter was inserted at a 45° angle to a depth of 10 cm while the other lysimeter was inserted vertically to a depth of 30 cm depth. The tension lysimeters were evacuated to 70 centibars prior to the day of sampling.

Shallow groundwater samples were collected from four wells located in the valley-bottom wetlands (Fig. 1). Shallow groundwater wells were constructed of 5 cm PVC tubing, were augured to a depth of 2 m and were screened for the full length from 30 cm below the soil surface. Two deep groundwater wells were collocated with shallow groundwater in the wetlands and were similar except that they were screened for only the lowermost 50 cm so as to collect only the deeper portion of groundwater (1.5–2 m below the soil surface). The riparian groundwater well was identical to shallow groundwater except that it was located in a riparian location which was not a wetland (Fig. 1). The riparian soil was well drained and had a thinner O horizon. Hyporheic samples were collected at two stream locations (Fig. 1) and consisted of slotted PVC pipes (5 cm diameter) inserted at a 45° angle to a depth of 30 cm in the stream bed. All samples from soil and groundwater locations were recovered using a hand-operated suction pump. Seep samples were collected manually at two seep locations from which one of the catchment tributaries originated (Fig. 1).

Throughfall and litter (or forest floor/O horizon) leachate sampling was performed following storm events. Throughfall sampling was performed at two locations (Fig. 1) and consisted of 1 L amber glass bottles that collected throughfall via a plastic funnel. A wire mesh was placed on the funnel to prevent entry of debris. Litter leachate collectors were placed at two locations (Fig. 1) and consisted of 1 L amber glass bottles connected to (via plastic tubing) plastic trays (~ 1 m^2) that contained the O horizon layer. These litter samplers were screened such that litter-leachate drained immediately into the amber glass bottles and no ponding occurred during or after the event. These samples were collected within 24 h of the storm event.

Sample processing and chemical analysis

All samples were filtered through a 0.45 μm filter paper (Millipore, Inc.) within 24 h of collection and stored at 4°C. A subsample for nutrients, cations, and anion analyses was stored in HDPE acid-rinsed bottles prior to analysis. Samples for DOM characterization were stored in amber glass bottles prior to UV and fluorescence measurements.

The Biogeochemistry Laboratory at SUNY-ESF, NY, which is a participant in the USGS QA/QC program, performed the following analyses: pH using a pH meter; Fe_{tot} using a Perkin-Elmer ICP-AEC Div 3300 instrument; NO_3^{-3} using a Dionex IC; NH_4^+ with an autoanalyzer using the Berthelot Reaction followed by colorimetric analysis; total dissolved nitrogen (TDN) using the persulfate oxidation procedure (Ameel et al. 1993) followed by colorimetric analysis on an autoanalyzer; and DOC using the Tekmar–Dohrmann Phoenix 8000 TOC analyzer. DON concentrations were computed as the difference between TDN and inorganic N (NO_3^-, NH^{4+}) (Inamdar and Mitchell 2007).

Characterization of DOM quality using spectrofluorometric indices

While DOC and DON concentrations and UV absorbance was determined for all samples, fluorometric scans and EEMs analyses were performed on only a selected subset of the samples. Optical indices used to characterize DOM quality are given in Table 1. The molar UV absorptivity of DOC at 254 nm was measured within 24 h after sample collection using 1 cm quartz window cuvette with a double beam Shimadzu UV-mini spectrophotometer (Shimadzu Inc.). SUVA (specific UV absorbance) provides a measure of aromaticity of DOM (Weishaar et al. 2003) and was computed by dividing the UV absorbance at 254 nm (m^{-1}) by the concentration of DOC (mg C L^{-1}) (Hood et al. 2006). Aromaticity increases with increasing values of SUVA (Weishaar et al. 2003). Since iron (Fe) absorbs light at 254 nm, elevated concentrations of Fe (>0.5 mg L^{-1}) can lead to incorrect SUVA values (Weishaar et al. 2003). We verified this by checking our SUVA values against the sample Fe values (corresponding DOC values were also checked). Most of our data (more than 99%) had low Fe values ($\ll 0.5$ mg L^{-1}). A few (less 0.5%) which had both high SUVA (>5) and high Fe values

Table 1 Definition and significance of the suite of DOM optical indices used in this study

DOM quality index	Reference	Definition and significance
UV		
Specific UV absorbance ($SUVA_{254}$) [$L\ mg\ C^{-1}\ m^{-1}$]	Weishaar et al. (2003)	UV absorbance at 254 nm divided by DOC concentration in mg C L^{-1}; provides a measure of aromaticity of DOM. High values of SUVA indicate more aromatic material
Absorption coefficient	Green and Blough (1994)	(UV absorbance at 254 nm) × 2.303 × 100
a_{254} [m^{-1}]		Measure of aromaticity of DOM
Slope ratio	Helms et al. (2008)	Ratio of the slope of the shorter UV wavelength region (275–295 nm) to that of the longer UV wavelength region (350–400 nm); Can be used as a proxy for molecular weight (MW);
S_R		S_R decreases with increasing MW
Fluorescence		
Humification index	Ohno (2002)	$HIX = \sum I435–480/(\sum I300–345 + \sum I435–480)$
HIX		Used to characterize humification status of DOM; ranges from 0 to 1 and increases with increasing degree of humification
Fluorescence index	McKnight et al. (2001)	Ratio of fluorescence intensities at 470 and 520 nm at excitation of 370 nm;
FI		Used to distinguish between terrestrial and microbial sources of DOM;
		Terrestrial or allochthonous DOM: 1.2–1.5; microbial or autochthonous DOM: 1.7–2.0
% C3	Cory and McKnight (2005)	Component 3 in Cory and McKnight (2005) PARAFAC model
		Indicates DOM of microbial or planktonic origin
% C5	Cory and McKnight (2005)	Component 5 in Cory and McKnight (2005) model
		Indicates higher plant-derived (versus microbial) organic matter and is also a proxy for aromatic organic matter
% Protein-like fluorescence	Cory and McKnight (2005)	Sum of % tyrosine (C13) and % tryptophan (C8) components from the Cory and McKnight (2005) model. Indicates protein-like DOM moieties and has been found to be a strong indicator of bioavailable DOM (Fellman et al. 2008, 2009)
Tyrptophan:tyrosine	Cory and McKnight (2005), Fellman et al. (2008)	Ratio of the C8 and C13 components from Cory and McKnight model
		High values of the ratio would indicate less degraded "fresher" proteins while low values would suggest degraded "older" proteins

($>0.5\ mg\ L^{-1}$) were not included in this analysis. In addition to SUVA, the absorption coefficient at 254 nm (a_{254} in m^{-1}) was also calculated following the procedures of Green and Blough (1994). The a_{254} also provides a measure of aromaticity but without normalization to C (Helms et al. 2008). Another UV index, the spectral slope ratio, S_R was also calculated as the ratio of the slope of the shorter UV wavelength region (275–295 nm) to that of the longer UV wavelength region (350–400 nm) (Helms et al. 2008) and was obtained using linear regression on the log-transformed spectral ranges (Yamashita et al. 2010). The spectral slope ratio, S_R, is inversely related to the molecular weight of DOM (Helms et al. 2008).

Three-dimensional fluorescence scans were collected on a Fluoromax-P spectrofluorometer (Horiba Jobin–Yvon Inc.) and corrected for the instrument bias with manufacturer provided correction files. A daily lamp scan, cuvette check and Raman water scan were run to ensure instrument stability. Scans were collected in S/R mode (ratio) for excitation wavelengths between 240 and 450 nm at 10 nm intervals and emission wavelengths between 300 and 550 nm at 2 nm intervals. The integration time for sample collection was set to 0.25 s along with bandpass of 5 nm each for excitation and emission ranges. Subsequently a Raman water blank was subtracted from each scan and resulting EEMs were

Raman-normalized using the area under the curve of water Raman peak at excitation 350 nm (Cory and McKnight 2005). To avoid inner filter effects, samples were diluted according to Green and Blough (1994). In addition, the inner filter correction proposed by McKnight et al. (2001) was also applied. The EEMs scans were fitted to the 13-component PARAFAC model developed by Cory and McKnight (2005). The advantage of using this existing model as opposed to developing our own PARAFAC model was that a large sample size was not required, and a greater amount of variation in the DOM source and quality was likely to be identified (Miller and McKnight 2010).

To elucidate differences in the quality of DOM, four of the 13 (Cory and McKnight 2005) components were selected for further evaluation. Component 3 (% C3) is considered to represent DOM of microbial or planktonic origin (Cory and McKnight 2005) while component 5 (% C5) is assumed to indicate DOM derived from higher plants (terrestrial origin) and has also been used as a proxy for aromatic material (Cory and McKnight 2005). Percent protein-like fluorescence was calculated as the sum of % values for tryptophan-like fluorescence (component 8) and tyrosine-like (component 13) from the Cory and McKnight (2005) model. The sum of these two protein components has been found to be a strongly correlated with bioavailable DOM (Fellman et al. 2008, 2009). In addition, we also computed the ratios of the % tryptophan-like and % tyrosine-like components. Tryptophan is considered to be less degraded peptide material and contains intact proteins while tyrosine represents more degraded peptide material (Fellman et al. 2009; Yamashita and Tanoue 2004). Thus, high values of the tryptophan and tyrosine ratio would indicate less degraded, "fresher" proteins while low values would suggest degraded "older" proteins. The fluorescence index (FI) was calculated using the ratio of fluorescence emission intensities at 470 and 520 nm at an excitation wavelength of 370 nm (Cory and McKnight 2005). McKnight et al. (2001) have used the FI to differentiate between DOM derived from higher terrestrial plants (FI: 1.3–1.4) versus microbial or planktonic sources (FI: 1.7–2.0). Lastly, the humification index (HIX) was calculated using the normalized HIX equation of Ohno (2002) which reduces the variation introduced by changes in DOM concentration (Ohno 2002). The HIX values for this modified equation range from 0 to 1 with higher values indicating a greater degree of humification/degradation of DOM (Ohno 2002).

Statistical analysis

General descriptive statistics including maxima, minima, average and median values and standard deviation was performed on DOC and DON concentrations and DOM quality parameters (DOC:DON, SUVA, a_{254}, HIX, S_R, % C3, % C5, % protein-like fluorescence, FI and tryptophan: tyrosine ratio) and compiled in the form of box plots. Significant ($p < 0.05$) differences between the watershed compartments for DOM concentrations and quality parameters were determined using the Tukey's parametric test. To further evaluate the differences among watershed compartments and to identify the key parameters influencing these differences a principal component analysis (PCA) was performed using DOM concentrations and quality indices. The compartments were plotted in the PCA space defined by the first two components along with the loadings for the two components. PCA was performed using JMP version 8.0 statistical software.

Correlations between DOM concentrations and quality indices were determined using the Pearson correlation coefficient. Results are reported for significance levels of $p < 0.10$ and all statistical analyses were performed using the statistical software JMP version 8.0.

Results

DOM concentrations and quality across watershed compartments

Dissolved organic matter (DOM) concentrations revealed pronounced patterns across the watershed sources of DOM (Figs. 2, 3). Significant ($p < 0.05$) differences among compartments for DOM are indicated in Table 2. Excitation-emission matrices for six of the 10 watershed compartments are presented in Fig. 4 to visually illustrate the changes in fluorescence across watershed sources. Both DOC and DON concentrations decreased from surficial sources (throughfall and litter) to deep groundwater sources but the decrease in DOC was sharper than

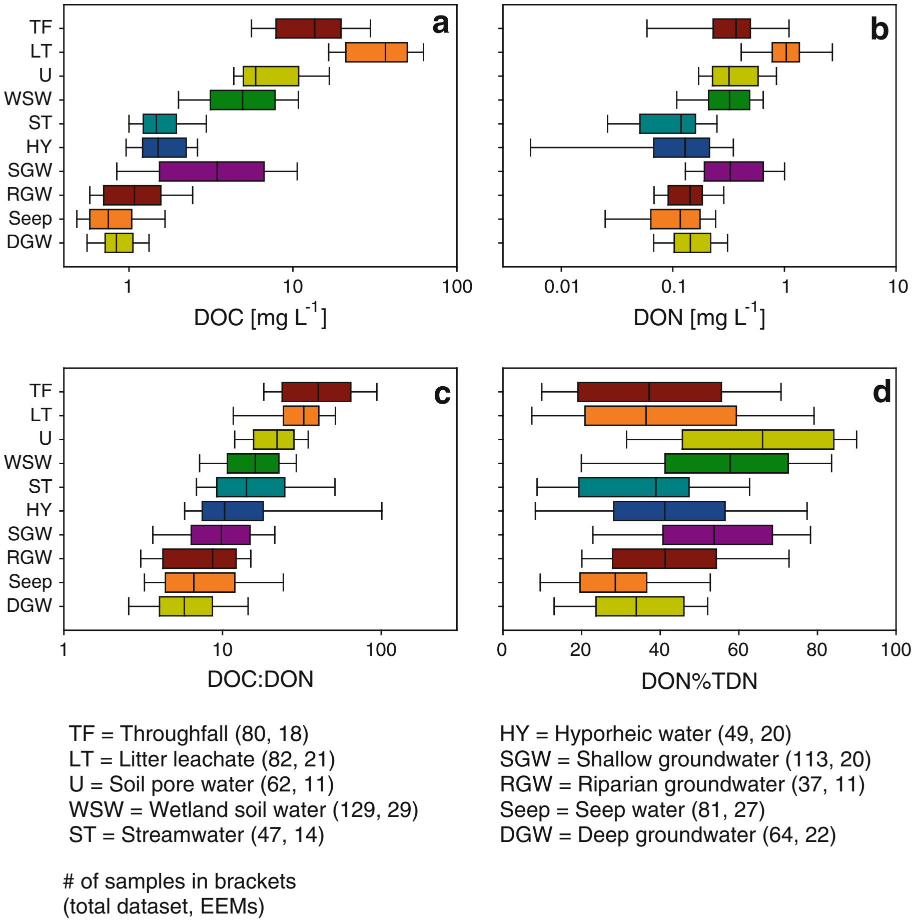


Fig. 2 Concentrations of: **a** DOC (mg L^{-1}); **b** DON (mg L^{-1}); **c** DOC:DON ratios and **d** DON as a % of total dissolved N (TDN) for the various watershed sources (*line* median, box upper and lower bound = 25% and 75%, whiskers = 10% and 90%). Sample numbers are indicated within *brackets* in the legend with the first value indicating the number of samples used for DOM concentrations and UV while the second value is the number of EEMs performed. Note the DOC and DON concentrations are plotted on logarithmic scale

DON. Median DOC concentrations (Fig. 2a) were highest in litter (37 mg L^{-1}) followed by throughfall (14 mg L^{-1}). This was followed by a large decrease and much lower concentrations in tension soilwater (5.9 mg L^{-1}), wetland soilwater (4.1 mg L^{-1}) and shallow groundwater (3.5 mg L^{-1}). Median concentrations were lowest in seeps (0.75 mg L^{-1}) whereas concentrations in deep and riparian groundwaters were only marginally higher (0.84 and 1.1 mg L^{-1}, respectively). Similar to DOC, median DON concentrations were also highest in litter (1.0 mg L^{-1}) followed by throughfall (0.37 mg L^{-1}) (Fig. 2b). Following these two sources, median concentrations of DON were comparable for shallow groundwater (0.33 mg L^{-1}), tension soilwater (0.32 mg L^{-1}) and wetland soilwater (0.30 mg L^{-1}). Lowest DON concentrations were recorded in Seep (0.12 mg L^{-1}) and streamwater (0.12 mg L^{-1}) and only marginally higher in riparian and deep groundwaters (both 0.14 mg L^{-1}) and hyporheic water (0.13 mg L^{-1}). Median DOC:DON ratios (Fig. 2c) were highest for throughfall (40) followed by litter (33). Median DOC:DON ratios then decreased systematically from litter to deep groundwater (6). Largest variability in median DOC:DON ratio was observed for the stream and hyporheic waters. To provide some perspective on how DON compared to dissolved inorganic nitrogen at our watershed site, we also include a plot of DON as a % of total dissolved N (Fig. 2d). % DON values were highest in tension soilwater

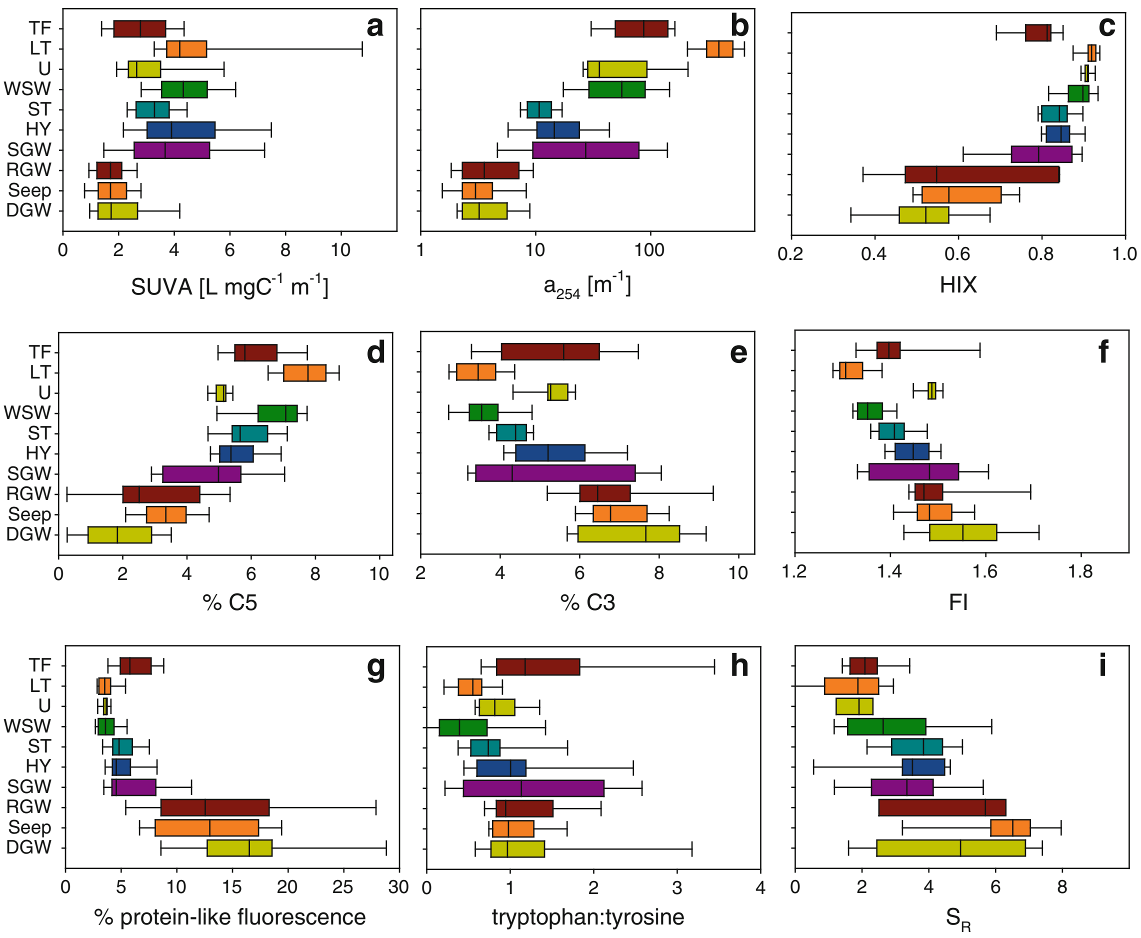


Fig. 3 Values for DOM quality indices for various watershed sources (*line* = median, box upper and lower bound = 25% and 75%, whiskers = 10% and 90%). These indices include: Specific UV absorbance (SUVA), absorption coefficient a_{254}, humification index (HIX), Cory and McKnight (2005) model components: % C5, C3 and protein-like fluorescence, fluorescence index (FI), spectral slope ratio S_R, and ratio of tryptophan-like and tyrosine-like components. *Note* only **b** has a logarithmic *x*-axis

(~70%) and decreased slightly with soil depth, resulting in a minimum for the seep.

While there was a large range in SUVA values (~1 to more than 10, Fig. 3a) highest median values for SUVA were recorded for wetland soilwater (4.3) followed by litter (4.2), hyporheic (3.9) and shallow groundwater (3.7). Median SUVA values were slightly lower in stream water (3.3) and much lower in tension soilwater (2.7). The lowest median SUVA values were observed in the groundwater sources—seep (1.7), riparian groundwater (1.7) and deep groundwater (1.7). Median SUVA values for throughfall were lower than that for litter but greater than that of the groundwater compartments. Following the linear model developed by Weishaar et al. (2003), litter leachate with a median SUVA value of 4.2 represented an aromatic C content of 31%. There was considerable variability in SUVA for the litter, hyporheic and shallow groundwater components.

Compared to SUVA, the other aromatic indices—a_{254} and % C5 displayed a more pronounced and systematic trend across the watershed sources (Fig. 3b, d). Median values for both a_{254} and % C5 were highest in litter (391 and 7.8, respectively) and decreased in the order of: wetland soilwater (40 and 7.1, respectively), shallow groundwater (22 and 4.6, respectively) and deep groundwater (3.2 and 1.8, respectively). Median values of a_{254} and % C5 for throughfall were less than litter. Intermediate values were observed for the other watershed compartments.

Table 2 Results from Tukey test indicating the significant ($p < 0.05$) differences among the watershed sources

	DOC					DON		SUVA				a_{254}					HIX			
TF		B					B				D		B					B		
LT	A					A		A				A					A			
U			C				B			C	D		B	C			A			
WSW			C	D			B	A	B				B	C			A			
ST				D	E		B		B	C	D				D	E	A	B		
HY				D	E		B	A	B						D	E	A	B		
SGW				D	E		B	A	B	C				C	D			B		
RGW				D	E		B				D				D	E			C	
Seep					E		B				D					E			C	
DGW					E		B				D					E				D

	% C5						% protein-like fluorescence			% C3				FI				S_R				trp/tyr[a]		
TF		B	C						C		B	C			B	C				C	D	A		
LT	A								C				D				D				D			C
U			C	D					C		B	C	D	A	B					C	D	A	B	C
WSW	A	B							C							C	D			C			B	C
ST		B	C						C			C	D		B	C			B	C		A	B	C
HY			C	D					C			C			B				B	C		A	B	C
SGW				D					C			C			B				B	C		A		
RGW					E		A	B		A	B			A	B			A	B			A	B	C
Seep					E	F		B		A				A	B			A				A	B	C
DGW						F	A			A				A				A	B			A	B	

Sources followed by the *same letters* vertically within a column are not significantly different. Watershed sources sampled were throughfall (TF), litter (LT), tension soil water (U), zero-tension wetland soil water (WSW), stream water (ST), hyporheic water (HY), shallow groundwater (SGW), riparian groundwater (RGW), groundwater seeps (Seep), and deep groundwater (DGW)

[a] Ratio of % tryptophan to % tyrosine components

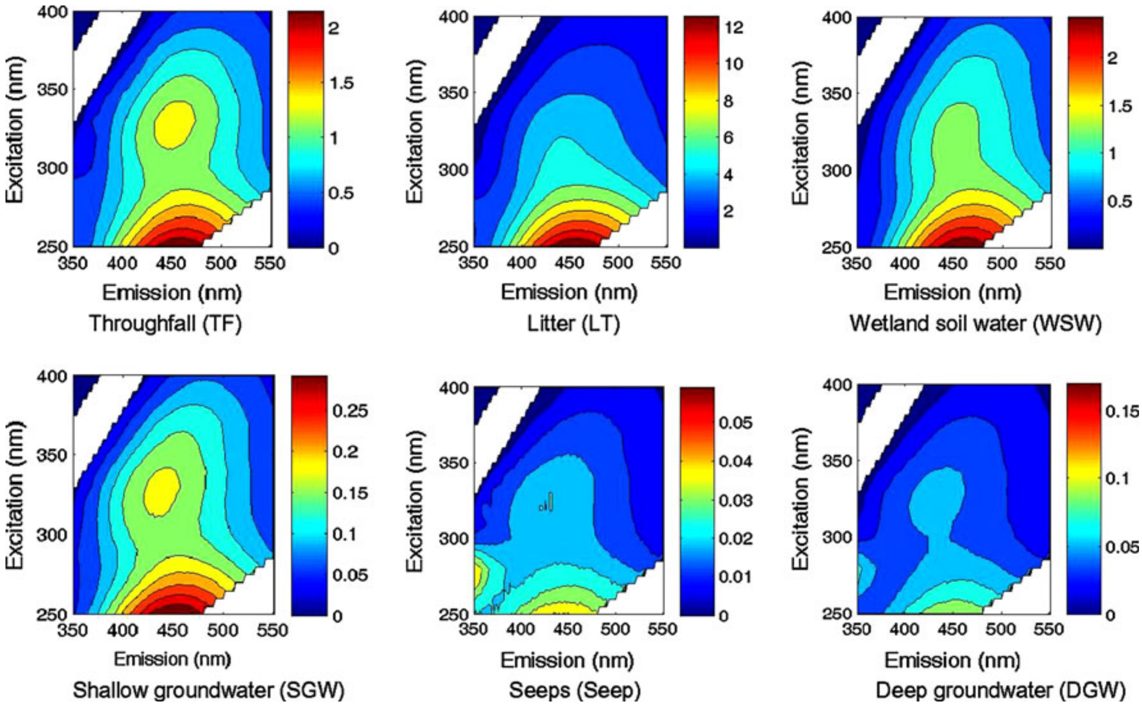


Fig. 4 Excitation-emission matrices (EEMs) for selected watershed sources highlighting the shift in fluorescence. Throughfall and litter samples correspond to event of October 25, 2008, while other samples were collected during manual grab sampling on October 10, 2008. Fluorescence intensity is in Raman Units

The values for SUVA, a_{254}, and % C5 all show that DOM from riparian groundwater was less aromatic compared to shallow groundwater suggesting that the wetland locations had more aromatic DOM.

While the overall trend for HIX was similar to a_{254} and % C5 there was a sharper drop in HIX for the seep and deep groundwater compartments (Fig. 3c). HIX provides a measure of the humification status of DOM and median values for HIX were highest in litter leachate (0.92) followed by wetland soilwater (0.88), shallow groundwater (0.78) and deep groundwater (0.52). Median HIX values for stream and riparian groundwater were 0.84 and 0.55, respectively. Median HIX values for throughfall were lower than those for litter and the soil water components suggesting that DOM in throughfall was not as degraded.

Indices that have been used as a proxy for "microbial" sources of DOM (% C3 and FI) followed a trend exactly opposite to that observed for the aromatic indices (a_{254} and % C5) (Fig. 3e, f). In contrast to a_{254} and % C5, both % C3 and FI increased from litter to deep groundwater. As reported in Table 1, FI values in the range of 1.2–1.5 have been used to represent DOM from vascular plants (or allochthonous sources) whereas values of 1.7–2.0 represent microbial or planktonic DOM (or autochthonous DOM; McKnight et al. 2001). Both % C3 and FI were lowest in litter (3.4 and 1.31, respectively) and increased in the order as: wetland soilwater (3.49 and 1.37), shallow groundwater (4.52 and 1.49) and deep groundwater (7.66 and 1.55). Compared to deep groundwater, median values for these two indices were slightly lower in riparian groundwater (6.45 and 1.47) and seep water (6.78 and 1.48). Values for streamwater (% C3 and FI: 4.39 and 1.41, respectively) and hyporheic water (% C3 and FI: 5.21 and1.45, respectively) were intermediate between wetland soilwater and deep groundwater. Median values for both % C3 and FI in throughfall were greater than the corresponding values for litter.

The trend for % protein-like fluorescence was similar to the microbial indices (Fig. 3g) % C3 and FI. Percent protein-like fluorescence was lowest in litter (3.52) and increased in the order as: wetland soilwater (3.97), shallow groundwater (4.59) and deep groundwater (16.5). A sudden increase in % protein-like fluorescence can be noted between shallow and riparian groundwaters. Compared to the surficial (throughfall and litter) and soilwater DOM, the variability in % protein-like fluorescence was larger for the deeper groundwater sources. Highest value for the tryptophan/tyrosine ratio was observed for throughfall and lowest for wetland soilwater and litter (Fig. 3h). The soil and groundwater compartments were fairly similar in values for this ratio.

The values of S_R were lowest for litter leachate (1.87) and increased in the order: wetland soilwater (2.63) < shallow groundwater (3.35) < deep groundwater (4.95). Highest S_R values were found in seeps (6.51) and riparian groundwaters (5.69). S_R values for stream (3.68) and hyporheic waters (3.51) were intermediate between shallow and deep groundwaters. Overall, these values suggest that molecular weight of DOM was highest near the soil surface and decreased for deeper watershed sources. The values of S_R for throughfall were slightly greater than litter indicating that molecular weight of DOM was lower in throughfall. The median value of S_R for riparian groundwater was considerably greater than that for shallow groundwater indicating a lower molecular weight of DOM at the riparian location.

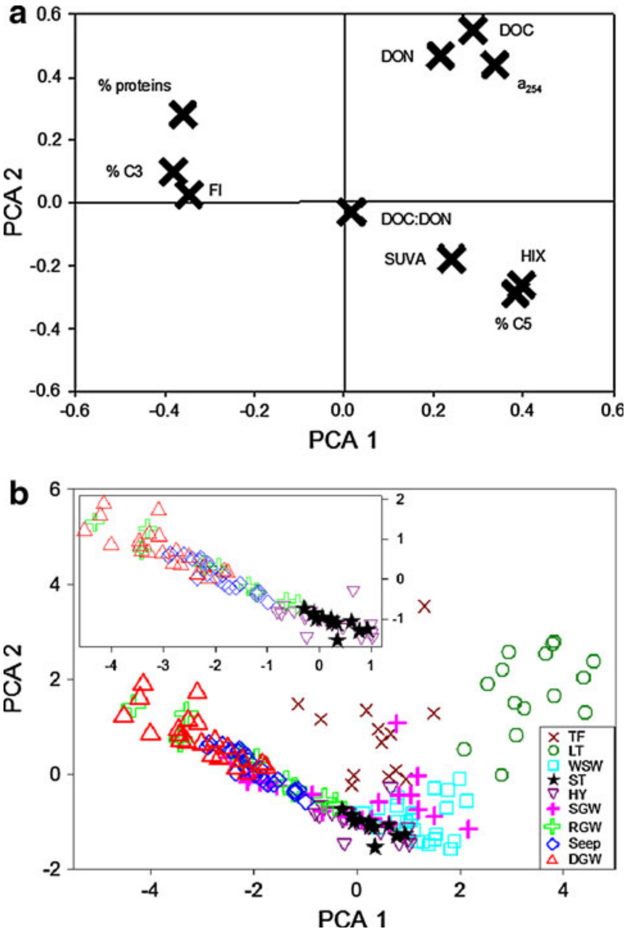
Principle component analysis (PCA) on DOM concentrations and quality metrics

The PCA analyses for DOM with the property–property plots for first and second factor loadings are presented in Fig. 5a while the component scores for the two components are presented in Fig. 5b. DOM quality indices rather than DOM concentrations explained most of the variance among the watershed compartments. HIX, % C5 and % C3 explained 48% of the variance in the first principal component, with HIX and % C5 indicating positive and % C3 representing negative first factor loadings (Fig. 5a). DOC concentrations explained 14.4% of component 2 loadings followed by DOC:DON ratios explaining 10.2% of component 3 and SUVA explaining 9.6% of component 4 (only component 1 and 2 are plotted in Fig. 5). Watershed compartments displayed important differences in how they were distributed in PCA space (Fig. 5b). Data from litter leachate, throughfall, wetland soilwater and shallow groundwater were distributed as clusters with relatively similar variances along both component axes. Of these four compartments, litter showed the highest positive values for components 1 and 2, with slightly lower values for throughfall for both components 1 and 2, followed by wetland soilwater and shallow groundwater with lower values for component 2 but very similar values for component 1. In contrast to the clustered nature of these four compartments, deep and riparian groundwaters and groundwater seeps displayed a strong linear distribution with a much greater variance along the PC1 axis than PC2. Stream and hyporheic water samples were intermediate between these two patterns, with a linear trend along PC1 but more clustered distribution along PC2. The linear trend and the orientation of the groundwater sources on the PCA plot (see inset in Fig. 5b) suggest a strong influence of microbial and protein-like indices and a weak influence of the aromatic indices. In contrast, the spatial distribution of the clustered compartments revealed a greater influence of SUVA, HIX and a_{254} (aromatic and humic-like indices) on these watershed compartments.

Correlations between DOM concentrations and quality metrics

The intent for these analyses were: (a) to determine if DOC and DON concentrations were preferentially associated with any particular DOM quality pools (e.g., aromatic DOM or % protein-like fluorescence); (b) to investigate if the DOM quality indices corroborated each other; (c) to explore if any particular metrics yielded consistently strong or weak correlations and thus were more or less preferable than others; and (d) to investigate if correlations were weaker or stronger for any specific watershed source. While we only report correlations significant at $p < 0.10$ in Table 3, there were a few metrics for which the relationships were obvious but not significant at $p < 0.10$. DOC and DON concentrations were positively correlated across all watershed compartments except riparian and deep groundwaters, for which, there was no significant correlation. The highest correlation in DOC–DON concentrations was observed for tension soilwaters while the lowest values were observed for stream and seep samples. The correlations between a_{254} and DOC were stronger with positive relationships across all compartments except deep groundwater. Unlike a_{254}, the

Fig. 5 Principal component analyses (PCA) for DOM with **a** property–property plots for first (PCA1) and second (PCA2) factor loadings; and **b** component scores for the first two components



other humic-like/aromatic indices—HIX and % C5 did not show as strong a relationship with DOC concentration. The correlations between DOC and HIX were positive for six watershed compartments but negative in throughfall. The correlations between % C5 and DOC were positive for only two watershed sources. The "microbial" and protein-like indices (% protein-like fluorescence, % C3, and FI) all reported negative relationships with DOC concentration. Among these three indices, correlations between % protein-like and DOC were especially strong and negative for six of the ten watershed compartments. The relationship between DOC concentration and S_R was also strong and negative for five watershed compartments indicating that DOC was associated with the high MW DOM.

Similar to DOC, the correlations between DON and a_{254} were also positive for nine of the 10 watershed compartments, but compared to the relationships to DOC concentrations, the correlations between DON and a_{254} were generally weaker (lower correlation coefficients). For the other humic-like/aromatic indices—HIX, and % C5, the correlations with DON were positive for two and one watershed compartment, respectively. DON was positively correlated with % protein-like fluorescence in litter but negatively correlated in tension soil water, hyporheic and shallow groundwater compartments. With FI, DON was negatively correlated for only two compartments—hyporheic and shallow groundwater. A negative correlation between DON and S_R was also observed for throughfall, litter, and tension soilwater.

Specific ultra-violet absorbance (SUVA) revealed significant correlations for only a few watershed compartments. Surprisingly, SUVA which is a measure of aromatic DOM did not reveal strong correlation with the other humic and aromatic metrics (HIX and % C5). The correlations of SUVA with the

Table 3 Pearson correlation coefficients ($p < 0.10$) for DOM concentrations and quality indices for various watershed sources

	TF (80,18)[a]	LT (82,21)	U (62,11)	WSW (129,29)	ST (47,14)	HY (49,20)	SGW (113,20)	RGW (37,11)	Seep (81,27)	DGW (64,22)
DOC–DON	0.57	0.37	0.77	0.41	0.30	0.64	0.46		0.33	
DOC–a_{254}	0.59	0.62	0.94	0.84	0.99	0.57	0.85	0.49	0.79	
DOC–HIX	−0.53			0.38	0.68	0.65		0.79	0.59	0.43
DOC–% C5								0.81	0.45	
DOC–% protein-like				−0.47	−0.73		−0.52	−0.72	−0.62	−0.37
DOC–% C3			−0.83	−0.41			−0.76			
DOC–FI							−0.78			
DOC–S_R	−0.39	−0.74	−0.93	−0.51				−0.75		
DON–a_{254}	0.31	0.27	0.74	0.43	0.53	0.32	0.47		0.35	0.23
DON–HIX						0.60		0.53		
DON–% C5							0.63			
DON–% protein-like		0.62	−0.37			−0.36	−0.47		−0.58	
DON–% C3			−0.56		−0.57		−0.69		0.41	
DON–FI						−0.39	−0.69			
DON–S_R	−0.26	−0.63	−0.82							
a_{254}–HIX				0.43	0.80			0.54	0.57	−0.40
a_{254}–% C5									0.49	
a_{254}–% protein-like	−0.36			−0.51	−0.71		−0.48		−0.39	0.36
a_{254}–% C3			−0.85	−0.51	−0.76		−0.75			
a_{254}–%FI							−0.74			
a_{254}–S_R	−0.44	−0.78	−0.86	−0.58			−0.47	−0.61		
SUVA–HIX	0.61									−0.54
SUVA–% C5										
SUVA–% protein-like	−0.49			−0.37				0.61		
SUVA–% C3	−0.40			−0.65						
SUVA–FI										
SUVA–S_R			−0.81	−0.60						
S_R–HIX								−0.87		
S_R–% C5						−0.57		−0.73		
S_R–% protein-like							0.65	0.59		
S_R–% C3				0.63			0.69			
S_R–FI										

Table 3 continued

	TF (80,18)[a]	LT (82,21)	U (62,11)	WSW (129,29)	ST (47,14)	HY (49,20)	SGW (113,20)	RGW (37,11)	Seep (81,27)	DGW (64,22)
HIX–% C5	0.70	0.47	0.74	0.46	0.63	0.56	0.86	0.96	0.69	0.83
HIX–% protein-like	−0.63	−0.95	−0.84	−0.84	−0.90	−0.76	−0.73	−0.92	−0.93	−0.89
HIX–% C3	−0.77				−0.50	−0.69	−0.37			0.37
HIX–FI		−0.38				−0.42	−0.50	−0.58	0.37	0.36
% protein-like–% C5	−0.42	−0.60	−0.59	−0.44	−0.75	−0.63	−0.49	−0.94	−0.66	−0.67
% protein-like–% C3				0.44			0.70			−0.37
% protein-like–FI		0.50					0.60	0.67		−0.47

Watershed sources include: throughfall (TF), litter (LT), tension soil water (U), zero-tension wetland soil water (WSW), stream water (ST), hyporheic water (HY), shallow groundwater (SGW), riparian groundwater (RGW), groundwater seeps (Seep), and deep groundwater (DGW)

[a] Number of samples used for DOM concentrations and UV, followed by number of samples analyzed for EEMs

% protein-like fluorescence, % C3 and FI metrics were also mixed. Similarly, significant correlations between S_R and other DOM quality metrics were observed for only a few watershed compartments. S_R was positively correlated with % protein-like fluorescence in shallow and riparian groundwater sources indicating that the protein-like fluorescence for these sources were associated with low molecular weight DOM. HIX produced the strongest correlations among all DOM metrics. HIX was positively correlated with % C5 across all sources, suggesting that the humified DOM material was also aromatic. HIX also indicated a very strong inverse relationship with % protein-like fluorescence across all compartments, indicating that DOM with protein-like fluorescence was not highly humified or degraded. The relationship between HIX and % C3 and FI were negative for most compartments. Finally, as expected, % protein-like fluorescence revealed a strong negative relationship with % C5 across all compartments and a predominantly positive relationship (with the exception of deep groundwater) with the microbial metrics % C3 and FI.

Discussion

Changes in DOM concentrations and quality across watershed sources

Results from this study revealed marked changes in DOM concentration and composition among the watershed sources. Similar to previous studies (Michalzik et al. 2001); DOM concentrations in throughfall were lower than those measured for the litter layer. Furthermore, compared to litter, throughfall DOM was less aromatic and humic-like (as indicated by SUVA, a_{254}, and % C5). Simultaneously, the % protein-like fluorescence content of throughfall was also higher than litter and the S_R values suggested that DOM in throughfall was lower in molecular weight than that in litter. While not many studies have performed fluorescence analyses for throughfall DOM, previous results from sorptive fractionation and other analyses of DOM (Qualls and Haines 1991) corroborate our observations. These studies report that compared to the litter layer, throughfall DOM tends to be more hydrophilic, less aromatic, and may contain a greater proportion of

DOM constituents with lower molecular weight (Kalbitz et al. 2000; Qualls and Haines 1991).

A second, more pronounced, trend in DOM among the watershed sources was that surficial sources (e.g., throughfall, litter, and soil water) had high DOM and were more aromatic and humic-like while DOM originating from deeper sources in the watershed (e.g., seeps, riparian, and deep groundwaters) were low in DOM and aromatic content (Fig. 3). Furthermore, compared to surficial sources, DOM from deeper sources also revealed higher values for % C3, FI and % protein-like fluorescence. High concentrations of DOC and DON in surficial sources such as forest floor or litter layer have been well documented (Kalbitz et al. 2000; Michalzik et al. 2001). Many of these studies have also shown that DOM in forest floors or surficial soil horizons is substantially humic and aromatic (Aitkenhead-Peterson et al. 2003; Qualls and Haines 1991). Concentrations of DOM have been found to decrease dramatically as DOM percolates through the mineral soil profile and have been attributed to the preferential sorption of hydrophobic, humic, and/or aromatic C compounds (Jardine et al. 1989; Kaiser and Zech 1998, Ussiri and Johnson 2004). The decrease in DOM concentrations and aromatic or humic-like constituents for deeper sources in our study clearly suggests that sorption onto mineral surfaces may be a factor. We hypothesize that watershed sources that originated from longer or deeper hydrologic flow paths had greater contact with the mineral soil surfaces thus resulting in lower DOM concentrations and aromatic content.

The increase in S_R values for DOM for deeper watershed sources in our study also suggests that molecular weight of DOM decreased along the hydrologic flow path through the mineral soil. This observation is consistent with previous studies that have found that DOM with high molecular weight (e.g., >1000 Da) and HIX values is preferentially sorbed to mineral soil while DOM with lower molecular weight (<1000 Da) is hydrophilic and remains in solution (Banaitis et al. 2006; Guo and Chorover 2003; Kaiser and Zech 2000). Recent work of Nguyen et al. (2010) found that molecular weight of DOM was positively correlated with HIX and hydrophobic fraction of DOM and negatively correlated with the protein-like fluorescence.

With the decrease in molecular weight of DOM, our data also revealed a simultaneous increase in amounts of % C3, FI and % protein-like DOM. % C3 and FI have been used as proxies for “microbial” DOM with FI being typically used to differentiate between DOM from microbial or planktonic origins (or autochthonous DOM, FI: 1.7–2.0) versus that from higher terrestrial plants (or allochthonous DOM, FI: 1.2–1.5) (McKnight et al. 2001). Thus, while our % C3 values indicate a slight increase in the “microbial” fraction of DOM with soil depth, the FI values, even with the increase with depth, remained mostly within the range reported for vascular plants or DOM of allochthonous origin. Thus, while there may have been some microbial processing of DOM along the hydrologic flow paths, we hypothesize that the increase in the % C3 and % protein-like fluorescence was primarily due to the % decrease of the humic-like components by sorption. This shift in the DOM character and the importance of protein-like DOM for deeper watershed sources was especially highlighted in our PCA plot (Fig. 5). The clustering and location of the surficial watershed compartments in the PCA diagram clearly suggests that while the humic-like components were important in discriminating between surficial DOM sources, the protein-like fluorescence became important for deeper DOM sources.

Through high performance liquid chromatography for O horizon leachates, Yu et al. (2002) found that proteins and amino compounds could form large complexes with phenols or humic substances resulting in high molecular weight DOM and display a hydrophobic behavior. They further hypothesized that the complexed proteins and amino acids were more likely to be sorbed and the proteins remaining in solution were non-complexed or free amino acids and proteins. Qualls and Haines (1991) also indicated that the proteins remaining in solution (hydrophilic) typically exist as free amino acids, peptides and proteins. Following the rationale of Yu et al. (2002) and our observations of increasing protein-like moieties and decreasing molecular weight of DOM with soil depth, we hypothesize that the protein-like fluorescence we observed in groundwater are likely free forms of the proteins. Vazquez et al. (2007, 2010) also concluded that proteinaceous DOM associated with groundwater was of low molecular weight.

The tryptophan- and tyrosine-like fluorescence determined from EEMs has also been used to characterize the degradation status of proteins and amino compounds (Fellman et al. 2008). Tyrosine is assumed to represent more degraded peptide material, while tryptophan is used a proxy for less degraded peptide material and intact proteins. Banaitis et al. (2006) evaluated these protein compounds in O-horizon and tree-leaf tissue extracts and found that the sorption potential for tryptophan was greater than tyrosine (52 vs. 29% sorption, respectively). Our observations on the ratio of % tryptophan to tyrosine (Fig. 3h) did not reveal any consistent trend with soil depth. Following the results of Banaitis et al. (2006), we expected that the tryptophan to tyrosine ratio would decrease with soil depth. However, other than the sharp decrease in the ratio from throughfall to litter, there was no decrease, rather, the ratio increased slightly with soil depth. Thus, the use of this ratio was inconclusive at our study site.

Studies have also reported that the proportion of C in DOM decreases much rapidly as it percolates through the soil profile resulting in decreasing DOC:DON ratio with soil depth (Qualls and Haines 1991). Some investigators have suggested that this is because the hydrophobic DOM (which is preferentially sorbed) contains more C while the hydrophilic compounds remaining in solution tend to be N rich (Kaiser and Zech 2000; Qualls and Haines 1991; Ussiri and Johnson 2004). In contrast, others have reported that N-rich DOM may also be hydrophobic and could be preferentially sorbed (McKnight et al. 1992; Yu et al. 2002). Yu et al. (2002) attributed the hydrophobic behavior of N-rich DOM moieties to complexation of N-rich amino compounds or proteins/peptides with polyphenols and/or humic substances. The decrease in DOC:DON ratio for deeper watershed sources (e.g., seep, RGW and DGW, Fig. 2) in our study is in line with the previous studies and suggests a greater loss of C-rich DOM with soil depth. This implies that DOC was indeed associated with the more humic or hydrophobic fractions of DOM. However, this implication was not necessarily supported by our correlation analyses (Table 3) which did not reveal that DOC had stronger correlations with humic material compared to DON. While the correlation coefficients between a_{254} and DOC were slightly higher than those for DON, the four indices (a_{254}, SUVA, HIX, and % C5) taken together did not provide conclusive evidence of higher C content in humic-like or aromatic DOM pools.

Influence of wetland and riparian conditions on DOM

This study also revealed some distinct differences between DOM from the well-drained riparian and the wetland groundwater sources (Figs. 2, 3). Despite the fact that both riparian and shallow groundwater wells sampled DOM from the same soil depth they revealed distinct differences in DOM concentrations and quality. Both DOC and DON concentrations were lower in the riparian groundwater versus the wetland groundwater. The DOC:DON ratio was also lower for the riparian versus the wetland position. In addition, DOM for wetland groundwater was much more aromatic and humic-like (note a_{254}, SUVA, % C5 and HIX values), of higher molecular weight (S_R) and with lower values of % C3 and % protein-like fluorescence compared to the riparian location (Figs. 2, 3). Following the Weishaar et al. (2003) model, the median SUVA value for the riparian groundwater of 1.8 translated into an aromatic content of 15% while the corresponding aromatic content of the shallow wetland groundwater came to 27% (median SUVA value of 3.7).

High concentrations of DOM in wetlands have been well recognized (Fellman et al. 2008; Hagedorn et al. 2001; Kalbitz et al. 2000). Many of these studies have also shown that compared to uplands, wetland DOM is more humic, aromatic (high SUVA values) and refractory (high HIX) (Fellman et al. 2008; Geller 1986; Kalbitz and Geyer 2002). The high concentrations and humic form of DOM in anoxic wetland soils has been attributed to low and inefficient decomposition of organic matter (Mulholland et al. 1990) and the release of DOM due to reductive dissolution of Fe and Al oxides (Hagedorn et al. 2001). We hypothesize that the well drained nature of the riparian location in our watershed likely promoted greater sorption of DOM on mineral surfaces and thus resulted in the lower aromatic and humic-like DOM observed for the riparian versus the wetland groundwater.

Implications for bioavailability of DOM

Previous work has shown that DOM with low humic-like and aromatic content (e.g., consisting of free peptides, proteins, amino acids, etc.) is more bioavailable (Kaushal and Lewis 2005; Keil and

Kirchman 1994; Qualls and Haines 1992; Maie et al. 2006). Fellman et al. (2008, 2009) performed microbial incubation assays and found that bioavailable DOC was inversely related to SUVA and DOC:DON ratio, but directly proportional to % protein-like florescence for wetland soils in Alaska (Table 4). Similarly, Balcarczyk et al. (2009) also noted the strong relationship between % protein-like fluorescence and bioavailable DOM (BDOM). While we do not have results from incubation assays in our study, the relationships between optical indices and BDOM from previous studies can provide some insights into the relative bioavailability of DOM among the various watershed sources.

The increasing % of protein-like fluorescence (Fig. 3g) from surficial to deeper watershed sources (LT < WSW < HY < ST < SGW < RGW < Seep < DGW) at our study site suggests that the % of BDOM increased for deeper watershed sources. This assessment is also supported by the trend in other indices like FI, % C3, a_{254}, and S_R. However, while the % of BDOM likely increased with soil depth, the DOM concentrations (Fig. 2) declined sharply. This indicates that despite the % increase in BDOM, the total amount of BDOM may remain the same or even decline for deeper watershed sources of DOM. A similar conclusion was reached by Balcarczyk et al. (2009) who found that the proportion of BDOM was highest in groundwater springs, but simultaneously cautioned that the total amount of BDOM may not be high because of the decrease in absolute amounts of DOM with soil depth.

A comparison of % protein-like fluorescence for wetland and riparian groundwaters (SGW and RGW, respectively, in Fig. 3g) at our site suggests that the proportion of BDOM in wetland groundwater was considerably less than that for riparian locations. Again, whether this trend extends to absolute BDOM, would be dictated by the difference in total DOM between the two locations. DOM concentrations in wetland groundwater at our site were much higher than the value for the riparian location (Fig. 2a, b). Geller (1986) reported that DOM from wetlands is

Table 4 Correlations between various DOM parameters reported for selected studies in literature

Reference	Site and sample type	Parameters correlated	Correlation
Balcarczyk et al. (2009)	Fairbanks, Alaska, USA; discontinuous permafrost soils, streamwater	FI vs. SUVA	Negative
		FI vs. DOC:DON	Negative
		BDOM vs. % protein-like fluorescence	Positive
Fellman et al. (2009)	Juneau, Alaska, USA; soil water	BDOC vs. % protein-like fluorescence	Positive
		Wetland: BDOC vs. SUVA	Negative
		Upland forest: BDOC vs. SUVA	None
		SUVA vs. humic-like fluorescence	Positive
Jaffé et al. (2008)	Multiple sites across US;	DOC vs. SUVA and FI	None
	Streamwater, lakes, estuaries	SUVA vs. FI	Negative
Nguyen et al. (2010)	Han River basin, Korea; streamwater	HIX versus molecular weight	Positive
		Protein-like fluorescence vs. molecular weight	Negative
		Hydrophobic fraction vs. molecular weight	Positive
Wu et al. (2007)	Ontario, Canada;	DOC vs. humic-like component	Positive
	Mixed deciduous forest with large wetland	DOC vs. molecular weight	Positive
		DOC vs. FI	Negative
		FI vs. molecular weight	Negative
Yamashita et al. (2010)	Tropical streams, Venezuela;	DOC vs. a_{350}	Positive
	Forest and grassy savanna	DOC vs. FI	Negative
		DOC vs. S_R	Negative
		DOC vs. $SUVA_{350}$	None
		FI vs. S_R	Positive
		FI vs. $SUVA_{350}$	Negative

recalcitrant and is largely unavailable for bacterial degradation. In contrast, Fellman et al. (2009) found that wetland soils produced more labile DOM than upland locations. Observations in the later study were, however, made in the glaciated, coastal rainforest region of Alaska which represents a very different climate, geology, and ecological conditions compared to our unglaciated site in the mid-Atlantic US.

Correlations between DOM concentrations and quality indices

Correlations between DOM concentrations and quality have been investigated in a number of recent DOM studies, a few of which are summarized in Table 4. Generally, the interest in exploring these relationships has been to: (a) determine if DOC or DON concentrations are associated with humic or non-humic DOM fractions; (b) if optical indices corroborate each other (i.e., SUVA and HIX following the same trend); and/or (c) to determine if the optical indices are related to ecological phenomena (e.g., bioavailable DOM from incubation assays, Fellman et al. 2008) so that these optical indices could then serve as valuable proxies for the phenomena of interest (Jaffé et al. 2008). Yamashita et al. (2010) found strong positive correlation between DOC and a_{350} and a negative relationship between DOC and S_R and DOC and FI. Others have reported that DOC:DON ratios are positively correlated to SUVA and inversely associated with FI, i.e., DOM rich in C is humic/aromatic while DOM rich in N is less humic and displays a stronger microbial behavior. Our results across multiple watershed compartments clearly corroborate some of these relationships but also suggest that the strength, and in a few cases the direction (positive or negative correlation) of the relationships, may change with watershed DOM sources. While we found that DOC was associated with humic-like or aromatic fractions of DOM we also found that DON was associated with the humic-like or aromatic moieties (though to a lesser extent). Interestingly, both DOC and DON were associated with higher molecular weight DOM fractions (correlation with S_R) for the surficial watershed compartments but the same relationship did not extend to deeper watershed sources. Removal of large molecular weight DOM compounds in surface soil (through sorption) could have been a contributing factor.

Dissolved organic carbon (DOC) was strongly and negatively correlated with % protein-like fluorescence (note the absence of this correlation for throughfall and litter compartments where humics dominate), but surprisingly, DON also revealed a strong negative correlation with % protein-like fluorescence. The correlation between % protein-like fluorescence and DON was, however, weaker than that between % protein-like fluorescence and DOC. Taken together, these results suggest that at our site—(a) both DOC and DON were associated with humic-like fractions, and (b) that DON was likely composed of many other constituents in addition to proteins. Another interpretation that could explain the negative correlation between DON and % protein-like fluorescence (as has been alluded to in the previous section) is that the EEM-derived % protein-like fluorescence represents only the free forms of proteins. It is very likely that DON at our site is also composed of a large number of combined proteins, but which are not being accounted by the protein-like fluorescence derived from EEMs. This interpretation would be more in line with the observations of Maie et al. (2006) and Yu et al. (2002) who found that a large proportion of DON was constituted by combined protein and amino compounds.

Our correlation results also reveal that some optical indices may yield more pronounced and consistent relationships than others. This is noticed in the strength and the number of significant correlation coefficients observed for a_{254} and HIX versus those reported for SUVA. Weak relationships for SUVA have also been reported in a number of previous studies (Fellman et al. 2009; Jaffé et al. 2008; Yamashita et al. 2010). Similarly, while SUVA did not yield a systematic gradient across compartments (Fig. 3) the other aromatic metrics (a_{254} and % C5) produced a more pronounced pattern. Finally, our results did not suggest that there were particular watershed sources that yielded stronger or a greater number of significant DOM correlations compared to others. We had expected that watershed sources or locations that had a greater potential for hydrologic mixing (intersection of multiple DOM sources and flowpaths) and therefore more complexity would yield weaker DOM relationships versus locations where DOM was less mobile and more intimately in contact with the substrate (e.g., tension soilwater). Clearly, our results suggest that DOM relationships for watershed compartments are complex and influenced by multiple factors.

DOM in watershed sources and its implications for DOM in stream runoff

Knowledge of DOM concentrations and quality for various watershed sources can be helpful for estimating stream DOM exports if the runoff sources to the stream are known. Alternately, if stream DOM concentrations are known, knowledge of DOM in watershed sources can assist in identifying (or verifying) the runoff sources through the use end-member mixing models (e.g., Inamdar and Mitchell 2006). Our observations show that surficial DOM sources (e.g., throughfall, litter, wetland soil water) were clearly more humic-like and aromatic with a lower content of protein-like fluorescence; and vice versa for the groundwater DOM sources. Thus, stream water during baseflow (which typically derives runoff from groundwater sources, e.g., Inamdar and Mitchell 2006) would be expected to be lower in humic-like and aromatic DOM. Not surprisingly then, DOM chemistry for the stream water presented in this study (Figs. 2, 3; sampled during non-storm periods) was considerably different from that observed for throughfall and litter. Stream water DOM (measured at the catchment outlet) was also different from the DOM chemistry of seeps from which the stream originated (Figs. 2, 3). This suggests that stream water DOM was likely influenced by contributions of wetland soilwater and other groundwater sources (SGW, RGW) and/or instream biotic processes (Brookshire et al. 2005) which altered stream DOM as runoff traveled from the seeps to the watershed outlet. In contrast to the baseflow DOM reported here, stream water DOM during storm events (Inamdar et al., in preparation) revealed large increases in aromatic and humic-like constituents which we attributed to runoff contributions from near-surficial sources such as throughfall and litter. Thus, knowledge of DOM for potential watershed sources can be very useful in explaining watershed exports of DOM under varying hydrologic conditions.

Conclusions

To our knowledge, this is the first study that has simultaneously evaluated DOM concentrations and quality for multiple watershed compartments over a two-year period using a suite of optical indices. Key conclusions that can be derived from this research are:

- There were pronounced differences in DOM concentrations and composition across watershed sources. Surficial watershed sources had higher DOM concentrations and were more aromatic and humic-like compared to DOM from deeper watershed sources. The proportion of % protein-like fluorescence increased for groundwater DOM sources suggesting a greater fraction of bioavailable DOM for these sources.
- Distinct differences in DOM quality were observed for wetland and riparian groundwater locations suggesting that saturated and/or anoxic conditions may influence the DOM quality at these landscape positions.
- Data from optical indices did not provide any conclusive evidence that indicated that DOC or DON was preferentially associated with humic-like or non-humic DOM constituents.
- Our results revealed that some optical indices may yield more pronounced and consistent patterns for watershed sources. Indices like a_{254}, % C5, HIX, % protein-like fluorescence and S_R provided important insights into DOM composition.

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